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SPECIFICATION FOR PAPAIN

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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SPECIFICATION FOR PAPAIN

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Indian Standard
SPECIFICATION FOR PAPAIN

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 26 April 1966, after the draft finalized by the Fruits and Vegetables Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 The dried latex obtained from the unripe fruit of *Carica papaya*, commonly known as papain, contains, among other things, proteolytic enzymes. It has been used largely in various industries, such as meat processing, tanning, textiles and pharmaceuticals. It is, therefore, necessary to ensure the quality of the product by preventing its adulteration and by having strict quality control which may be done on the basis of this Indian Standard.

0.3 This standard is one of a series of Indian Standards on processed fruits and vegetables.

0.4 No detailed specifications have been laid down for papain either within the country or abroad except in the British Pharmaceutical Codex (1954) which has fixed limits for its proteolytic activity. In the formulation of this standard due consideration has been given to other factors, such as different methods adopted for drying the product and its storage.

0.5 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes the requirements and methods of test for papain.

2. TYPES

2.1 The material shall be either in the granular or powdered form.

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*Rules for rounding off numerical values (*revised*).

3. GRADES

3.1 There shall be two grades of papain:

Grade 1, and
Grade 2.

4. REQUIREMENTS

4.1 General — Papain shall be prepared by drying the whole latex of unripe papaya fruits either in the sun or in mechanical driers. The fruits used for extracting latex shall be free from insect or fungal attack. The material shall be free from extraneous matter, such as starch, sugars and any other ingredient which is not the natural constituent of papain.

4.1.1 The material may contain sulphur dioxide, mixed as such or added in the form of sodium or potassium salts. The sulphur dioxide content in the material shall not exceed 3 000 ppm (*see* Table 1).

4.2 The requirements for the two grades of papain shall be as given below:

Grade 1 — The material shall possess a good, uniform and light to deep cream colour; it shall not possess any objectionable smell or odour other than the characteristic odour; and shall score not less than 85 points.

Grade 2 — The material shall possess reasonably good colour; shall be reasonably free from off-odour and objectionable smell; and shall score not less than 75 points.

The maximum and minimum number of points to be scored by different factors shall be as given below. Scoring shall be done according to the method prescribed in Appendix A.

	Maximum	*Minimum*	
		Grade 1	Grade 2
Colour	25	19	16
Odour	50	37	33
Absence of defects	25	19	16

4.2.1 *Colour*

Grade 1 — The material shall possess a good, creamy white, uniform colour and shall be free from discolouration.

Grade 2 — The material shall possess reasonably good colour, varying from light brown to brown.

4.2.2 *Odour*

Grade 1 — The material shall be practically free from any objectionable smell other than the characteristic odour.

Grade 2 — The material shall be reasonably free from objectionable smell other than the characteristic odour.

4.2.3 *Absence of Defects*

Grade 1 — The material shall be practically free from extraneous matter, such as sand, grit, insects, leaves and stalks of the plant.

Grade 2 — The material shall be reasonably free from extraneous matter, such as sand, grit, insects, leaves and stalks of the plant.

4.3 The product shall also conform to the requirements given in Table 1.

TABLE 1 REQUIREMENTS FOR PAPAIN

SL No.	CHARACTERISTIC	REQUIREMENT		METHODS OF TEST (REF TO APPENDICES)
		Grade 1	Grade 2	
(1)	(2)	(3)	(4)	(5)
i)	Moisture, percent by weight, *Max*	10·0	10·0	B
ii)	Total nitrogen, percent by weight, *Min*	8·5	7·5	C
iii)	True protein nitrogen, *Min*	50 percent of total nitrogen	50 percent of total nitrogen	D
iv)	Total ash, percent by weight, *Max*	11·0	11·0	E
v)	Acid insoluble ash, percent by weight, *Max*	0·2	0·2	F
vi)	Alcohol soluble extract, percent by weight, *Max*	30·0	30·0	G
vii)	Ether soluble extract, percent by weight, *Min*	8·0	8·0	H
viii)	Sulphur dioxide, ppm	3 000	3 000	J
ix)	Proteolytic activity (ml of 0·1 N NaoH), *Min*	6·0	4·5	K

5. PACKING AND MARKING

5.1 The material may ordinarily be packed in polyethylene bags, heat sealed or firmly tied. The bags shall be packed in air-tight or vacuumized containers.

5.1.1 When packed in vacuumized containers, the polyethylene bags shall not be heat sealed or firmly tied.

5.2 Marking — Each container shall be marked with the following particulars:

a) Name, type and grade of the material with the brand name, if any;
b) Name and address of the manufacturer;
c) Net weight of the contents of the can in grams;
d) Date of manufacture or code number indicating the date of manufacture, to be embossed; and
e) Manufacturing licence number.

6. SAMPLING

6.1 The method of drawing representative samples of the material and the criteria for conformity shall be as prescribed in **3** of IS : 2860-1964*.

7. TESTS

7.1 Tests for ascertaining conformity of the material to this specification shall be carried out as prescribed in relevant appendices referred to in col 5 of Table 1.

APPENDIX A

(*Clause* 4.2)

DETERMINATION OF THE GRADE OF THE PRODUCT

A-1. APPARATUS

A-1.1 White Porcelain Bowls — big enough to hold the contents of the container under examination.

A-1.2 Stainless Steel Spoons — table spoons.

A-2. PROCEDURE

A-2.1 Panel of Judges — Grades of the product shall be judged by a panel of three to five judges. All the judges constituting a panel shall be conversant with the factors governing the quality of the product. The containers shall be opened and the contents poured separately into white porcelain bowls. Each judge shall independently examine the contents from each of the containers and indicate scores for different characteristics.

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*Method of sampling and test for processed fruits and vegetables.

A-2.1.1 The judges shall consider the following characteristics: colour, odour, and absence of defects.

A-2.2 System of Scoring — The variations within each factor are so described that the scores may be ascertained for each factor and expressed numerically. The relative importance of each factor has been expressed numerically on a scale of 100. Each judge shall indicate the score for the individual factors, by the method described in Table 2, and record his observations in the Score Sheet (*see* P 23).

A-2.2.1 The scores as number of points indicated by the judges for the contents of each container for the three factors (*see* **A-2.1.1**) shall be recorded in a tabular form in the Score Card (*see* P 24) and the average score calculated for each factor with overall average for each container entered in the appropriate column (*see* Table 2 *and* **A-2.3.2**).

A-2.3 Ascertaining the Grade

A-2.3.1 *Consistency Among Judges* — To ascertain the consistency of judgement among the judges, the total score indicated by each of them for the contents of the same container shall be calculated by adding up the score for the various individual characteristics. If the difference between the maximum and the minimum of the total scores so obtained does not exceed ($K + 5$), where K is the number of judges, the scoring shall be deemed as consistent for the container under consideration. If the difference exceeds ($K + 5$), the score that is farthest from its immediate neighbour (the scores being arranged in one order), shall be discarded and the consistency among the remaining judges shall be examined.

A-2.3.2 When the consistency (*see* **A-2.3.1**) is thus established, the overall average scores indicated by the judges whose scoring has been found to be consistent shall be calculated for each container. The average score for each of the individual characteristic shall also be calculated by taking into account the corresponding scores as given by the same judges for the contents of the same container.

A-2.3.3 *Assignment of Grade* — In order to assign a grade for the contents of a container the following procedure shall be adopted:

Grade 1 — The score for each factor individually shall be not less than 75 percent of the maximum score obtainable, and the overall average score shall be not less than 85 points.

Grade 2 — The score for each factor individually shall be not less than 65 percent of the maximum score obtainable, and the overall average score shall be not less than 75 points.

TABLE 2 METHOD FOR INDICATING SCORES FOR PAPAIN

(*Clause* A-2.2)

ORGANOLEPTIC CHARACTERISTIC (1)	DESCRIPTION (2)	MAXIMUM NUMBER OF POINTS (3)
Colour	Good; creamy white; uniform; free from discolouration	25
	Colour not quite uniform tending towards slightly brown colour	19
	Reasonably uniform colour, a little discolouration to the extent that the product does not become unacceptable	16
	Dark brown to black colour; total discolouration	0
Odour	Characteristic odour; free from any objectionable smell	50
	Characteristic odour with a trace of sulphide smell	37
	Reasonably acceptable smell, that is not highly offensive	33
	Unbearable; objectionable smell	0
Absence of defects	Free from extraneous materials, such as sand, grit, insects, leaves and stalks of plants	25
	A few broken small pieces of leaves and/or a trace of grit	19
	A few (one or two) insects, very little sand, pieces of leaves and stalks pieces	16
	Lot of sand, grit, heavy insect infestation	0

APPENDIX B

[*Table* 1, *Item* (i)]

DETERMINATION OF MOISTURE

B-1. GENERAL

B-1.1 Either Method 1 (*see* **B-2**) or Method 2 (*see* **B-3**) may be used. Method 1 shall be the referee method.

B-2. METHOD 1

B-2.1 Apparatus — A glass flask (which can be heated by suitable means) provided with a reflux condenser discharging into a trap and connected to the flask. The connections between the trap and the condenser and the flask should be interchangeable ground glass joints. The trap serves to collect and measure the condensed water, and to return the solvent to the flask. The assembly of apparatus is shown in Fig. 1, and the various components are described in **B-2.1.1** to **B-2.1.6**.

B-2.1.1 *Flask* — A 500-ml flask made of hard resistance glass, well annealed and free from striae and similar defects as much as possible.

B-2.1.2 *Condenser* — Water cooled, glass reflux condenser, having a jacket approximately 400 mm long and an inner tube of 9·5 to 12·7 mm outside diameter. The tip of the condenser to be inserted in the trap may be ground off at an angle of 30° from the vertical axis of the condenser. When inserted into the trap, the tip of the condenser shall be 6 to 7 mm above the surface of the liquid in the trap after distillation conditions have been established.

B-2.1.3 *Trap or Receiver* — of 5-ml capacity, made of hard resistance glass, well annealed and free from striae and similar defects as much as possible; it may be provided with ground glass joints. It may have the shape, dimensions and tolerances as given in Fig. 1 and consist essentially of the upper chamber, together with the tube and ground joint leading to the flask and the graduated tube. The graduated portion shall have a capacity of 5 ml when filled to the highest graduation mark. The scale shall cover the range of 0 to 5 ml and shall be graduated in 0·1 ml. The graduation marks corresponding to each millilitre shall be numbered and carried completely round the tube. The half millilitre graduation marks shall be carried three-quarters-way round the tube and the remaining marks shall be carried half-way round the tube. The error at any indicated capacity shall not exceed 0·05 ml.

B-2.1.4 *Heat Source* — The source of heat may be either an oil bath or an electric heater provided with a sliding rheostat or other means of heat control. The temperature of the oil in the bath should not be very much higher than the boiling point of toluene.

B-2.1.5 *Copper Wire* — Long enough to extend through the condenser with one end twisted into a spiral. The diameter of the spiral should be such that it fits snugly within the graduated portion of the receiver and yet may be moved up and down.

B-2.1.6 *Tube Brush* — Tube brush should have a long wire handle to dislodge any adhering water droplets in the condenser. The brush may be of such thickness that it just slides into the condenser.

B-2.2 Reagents

B-2.2.1 *Potassium Dichromate-Sulphuric Acid Cleaning Solution*

B-2.2.2 *Toluene* — Saturated by shaking with a small quantity of water and distilled. Use the distillate for moisture determinations.

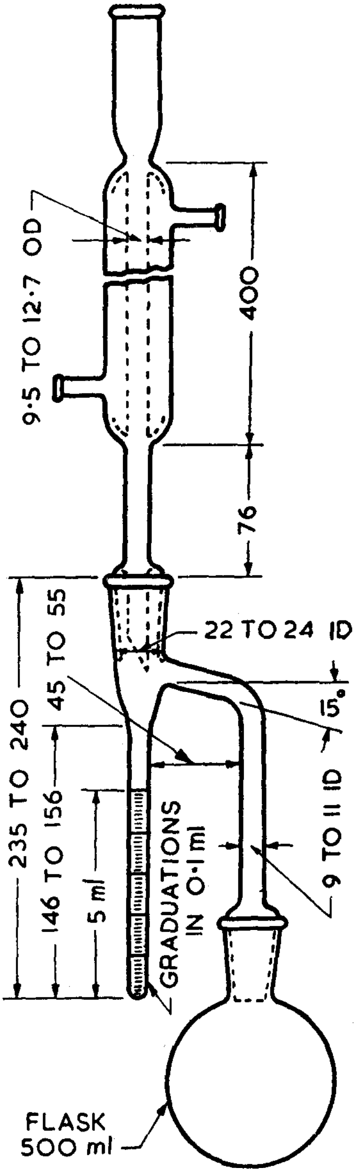

FIG. 1 APPARATUS FOR DETERMINATION OF MOISTURE BY TOLUENE DISTILLATION METHOD

B-2.3 Procedure

B-2.3.1 *Setting up the Apparatus* — Clean the entire apparatus with potassium dichromate-sulphuric acid cleaning solution to minimize the adherence of water droplets to the sides of the condenser and the receiver. Rinse thoroughly with distilled water and dry completely before using.

B-2.3.2 Place approximately 10 g of the ground material, accurately weighed, in the distillation flask, and add sufficient toluene (about 75 ml) to cover the sample completely; swirl to mix. Assemble the apparatus and fill the receiver with the solvent by pouring it through the condenser until it begins to overflow into the distillation flask. If necessary, insert a loose cotton plug in the top of the condenser or attach to it a small calcium chloride tube to prevent condensation of atmospheric moisture within the condenser tube. In order that the refluxing may be under control, wrap the flask and tube leading to the receiver with asbestos cloth. Heat the flask so that the distillation rate is about 100 drops per minute. When the greater part of water has distilled over, increase the distillation rate to about 200 drops per minute and continue until no more water is collected. Purge the reflux condenser occasionally during the distillation with 5-ml portions of toluene to wash down any moisture adhering to the walls of the condenser. The water in the receiver may be made to separate from toluene by moving the spiral copper wire up and down in the condenser and receiver occasionally, thus causing the water to settle at the bottom of the receiver. Reflux until the water level in the receiver remains unchanged for 30 minutes and then shut off the source of heat. Flush the condenser with toluene as required, making use of the spiral copper wire or the tube brush to discharge any moisture droplets. Immerse the receiver in the water at room temperature for at least 15 minutes or until the toluene layer is clear, and then read the volume of water.

B-2.4 Calculation

$$\text{Moisture content of the ground material, percent by weight} = \frac{100\,VD}{W}$$

where

V = volume in ml of water,

D = specific gravity of water at the temperature at which the volume of water is read, and

W = weight in g of the material taken for the test.

B-3. METHOD 2

B-3.1 Preparation of the Sample — Grind about 100 g of the sample so that it passes through 250-micron IS sieve (aperture 0·250 mm). If

250-micron IS sieve is not available, use ASA test sieve 250µ (same as the ASTM test sieve) or BS test sieve 60 or Tyler test sieve 60 (*see* IS : 460-1962*). Transfer this prepared sample to a well-stoppered glass bottle. Use this material for analysis.

B-3.2 Procedure — Weigh accurately about 5 g of the material (*see* **B-3.1**) in a tared dish having a diameter of at least 5 cm and depth of about 2 cm. Shake the dish until the contents are evenly distributed. Place the dish in an air-oven maintained at 105° ± 2°C and dry for at least 2 hours. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing until the difference between two successive weighings is less than 1 mg. Note the lowest weight.

B-3.3 Calculation

$$\text{Moisture, percent by weight} = \frac{100\, W_1 - W_2}{W_1 - W}$$

where

W_1 = weight in g of the dish with the material before drying,
W_2 = weight in g of the dish with the dried material, and
W = weight in g of the empty dish.

APPENDIX C

[*Table* 1, *Item* (ii)]

DETERMINATION OF TOTAL NITROGEN

C-1. REAGENTS

C-1.1 Concentrated Sulphuric Acid — Approximately 98 percent by weight and nitrogen-free.

C-1.2 Copper Sulphate

C-1.3 Potassium Sulphate or Anhydrous Sodium Sulphate — Nitrogen-free.

C-1.4 Sodium Hydroxide Solution — 50 percent by weight.

C-1.5 Standard Sulphuric Acid Solution — 0·1 N.

C-1.6 Standard Sodium Hydroxide Solution — Carbonate-free, 0·1 N.

---

*Specification for test sieves (*revised*).

C-1.7 Indicator Solution — Mix equal volumes of a saturated solution of methyl red in ethanol (95 percent by volume) and 0·1 percent solution of methylene blue in ethanol (95 percent by volume).

C-1.8 Sucrose — Anhydrous and pure.

C-2. APPARATUS

C-2.1 A recommended apparatus as assembled, is shown in Fig. 2. The apparatus consists of a round-bottom flask *A* of 1 000 ml capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube *B*. The other end of the bulb tube *B* is connected to the condenser *C* which is attached by means of a rubber tube to a dip tube *D* which dips into the liquid contained in a beaker *E* of 250 ml capacity.

C-3. PROCEDURE

C-3.1 Transfer a sample of papain, about 0·5 g to a 800-ml Kjeldahl flask. Add 25 ml of concentrated sulphuric acid, pouring this down the neck of the flask in such a way as to wash the powder into the body of the flask, and add 0·2 g of the copper sulphate. Gently rotate the flask so that the whole of the contents are well mixed.

C-3.2 Place the flask on a frame so that the neck is inclined at an angle of about 45° to the horizontal, and the bulb rests in the hole of an asbestos sheet so that the flame does not touch the flask above the level of the liquid. Heat to gentle boiling, and when frothing has ceased, add 10 g of potassium or anhydrous sodium sulphate. Boil the contents of the flask briskly until clear and free from yellowish colour; boil further for a period of one hour.

C-3.3 Allow the liquid to cool and wash down the sides with a fine jet of distilled water. Continue heating the contents of the flask for a further period of one hour. Allow the liquid to cool, dilute with approximately 200 ml of distilled water, transfer to a 1 000-ml flask, rinsing thoroughly, and add a few pieces of granulated zinc or a small amount of powdered pumice to prevent bumping. Add a suitable excess (usually 75 to 80 ml) of sodium hydroxide solution carefully down the neck of the flask to form a layer under the acid layer.

C-3.4 Fit the flask with a splash head and connect it to a condenser. Pipette a suitable excess (usually 50 ml) of standard sulphuric acid into a 250-ml beaker. Assemble the apparatus as shown in Fig. 2 taking care that the tip of the condenser extends below the surface of standard sulphuric acid in the beaker. Mix the contents of the flask by shaking and distil until all ammonia has passed into standard sulphuric acid. Detach the flask from the condenser and remove the source of heat. Rinse the condenser thoroughly with water into the beaker. Wash the tip tube carefully so that all traces of condensate are transferred to the beaker. Titrate the excess acid in the beaker with the standard sodium hydroxide solution using the indicator solution.

C-3.5 Carry out a blank determination by taking 0·5 g of sucrose in place of papain and by using the same quantities of reagents and the same conditions of test.

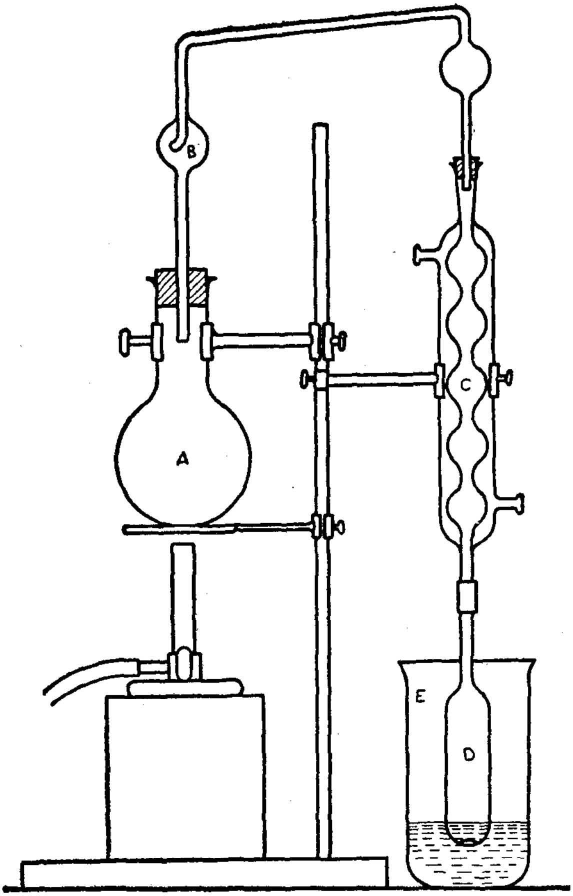

FIG. 2 APPARATUS FOR DETERMINATION OF NITROGEN

C-4. CALCULATION

C-4.1 Total nitrogen, percent by weight $= \dfrac{1{\cdot}4\,(A - B)\,N}{W}$

where

A = volume in ml of the standard sodium hydroxide required for the blank determination,

B = volume in ml of the standard sodium hydroxide required for the test,

N = normality of the standard sodium hydroxide, and

W = weight in g of the prepared sample taken for the test.

APPENDIX D

[*Table* 1, *Item* (iii)]

DETERMINATION OF TRUE PROTEIN

D-1. REAGENT

D-1.1 Trichloroacetic Acid Solution — 15 percent by volume.

D-2. PROCEDURE

D-2.1 Pipette 10 ml of the prepared sample into a 50-ml tared graduated flask and weigh accurately. Dilute to the mark with trichloroacetic acid solution and mix immediately. When the precipitate has settled, leaving a clear supernatant liquid, filter on a dry pleated 9-cm filter paper (Whatman No. 40 or its equivalent) into a dry flask. Wash the precipitate on the filter paper twice with the trichloroacetic acid solution.

D-2.2 Determine nitrogen in the washed precipitate and paper as prescribed in Appendix C.

D-3. CALCULATION

D-3.1 Obtain the percentage of true protein by multiplying the percentage of total nitrogen by the factor 6·38.

APPENDIX E

[*Table* 1, *Item* (iv)]

DETERMINATION OF TOTAL ASH

E-1. PROCEDURE

E-1.1 Weigh accurately about 2 g of the dried material in a tared porcelain, silica or platinum dish. Ignite with the flame of a Meker burner for about one hour. Complete ignition by keeping in muffle furnace at 600° ± 20°C until grey ash results. Cool in a desiccator and weigh. Ignite the dish again in the muffle furnace for 30 minutes, cool and weigh. Repeat this process until the difference in weight between two successive weighings is less than 1 mg. Note the lowest weight.

NOTE — Reserve the dish containing this ash for the determination of acid insoluble ash (*see* **F-2**).

E-2. CALCULATION

E-2.1 Total ash (on moisture-free basis), percent by weight $= \dfrac{100 (W_2 - W)}{W_1 - W}$

where

W_2 = the lowest weight in g of the dish with the ash,
W = weight in g of the empty dish, and
W_1 = weight in g of the dish with the dried material taken for the test.

APPENDIX F

[*Table* 1, *Item* (v)]

DETERMINATION OF ACID INSOLUBLE ASH

F-1. REAGENT

F-1.1 Dilute Hydrochloric Acid — 1 : 1.

F-2. PROCEDURE

F-2.1 To the ash reserved in the dish (*see* Note under **E-1**), add 25 ml of dilute hydrochloric acid. Cover with a watch-glass and heat on a water-bath for 10 minutes. Allow to cool and filter the contents of the dish through a Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from the acid and return it to the dish. Keep it in an electric air-oven maintained at 135° ± 2°C for about 3 hours. Ignite it in a muffle furnace at 600° ± 20°C for one hour. Cool the dish in a desiccator and weigh. Ignite the dish again in the muffle furnace for 30 minutes, cool and weigh. Repeat this process until the difference in weight between two successive weighings is less than 1 mg. Note the lowest weight.

F-3. CALCULATION

F-3.1 Acid insoluble ash (on moisture-free basis), percent by weight $= \dfrac{100 (W_2 - W)}{W_1 - W}$

where

W_2 = the lowest weight in g of the dish with the acid insoluble ash,

W = weight in g of the empty dish, and

W_1 = weight in g of the dish with the dried material (*see* W_1 in **E-2.1**).

APPENDIX G

[*Table* 1, *Item* (vi)]

DETERMINATION OF ALCOHOL SOLUBLE EXTRACT

G-1. REAGENT

G-1.1 Ethyl Alcohol — 95 percent by volume.

G-2. PROCEDURE

G-2.1 Place about 2 g of the material, accurately weighed, in a 100-ml graduated flask and fill to the mark with ethyl alcohol. Stopper and shake at intervals of 30 minutes for about 8 hours and allow to stand at the room temperature for 16 hours. Filter the extract through a dry filter paper and evaporate a 50-ml aliquot to dryness in a tared flat-bottom dish on a steam-bath and heat at 105° ± 1°C for three hours. Cool the dish and weigh. Heat at 105° ± 1°C for 30 minutes, cool and weigh again. Repeat the process of heating for 30 minutes, cooling and weighing until the loss in weight between two successive weighings is less than 1 mg.

G-3. CALCULATION

G-3.1 Alcohol soluble extract, percent by weight $= \dfrac{200\ (W_2 - W_1)}{W}$

where

W_2 = weight in g of the dish with the alcohol soluble extract,
W_1 = weight in g of the empty dish, and
W = weight in g of the ground material taken for the test.

APPENDIX H

[*Table* 1, *Item* (vii)]

DETERMINATION OF ETHER SOLUBLE EXTRACT

H-1. REAGENT

H-1.1 Diethyl Ether — anhydrous.

H-2. PROCEDURE

H-2.1 Extract 2 g of the material in a continuous extraction apparatus with diethyl ether for 18 hours. Remove ether by distillation, followed by blowing a stream of air into the flask on a boiling water-bath. Dry in an oven at 110° ± 1°C till the loss in weight between two successive weighings is less than 2 mg. Shake the residue with 2 to 3 ml of diethyl ether at room temperature, allow to settle and decant the ether. Repeat the extraction until no more residue dissolves. Dry the flask again until the loss in weight between two successive weighings is less than 2 mg. Note the lowest weight.

H-3. CALCULATION

H-3.1 Ether soluble extract, percent by weight $= \dfrac{100\ (W_1 - W_2)}{W}$

where

W_1 = weight in g of the flask with the non-volatile ether extract,
W_2 = weight in g of the flask with the ether insoluble residue after decantation, and
W = weight in g of the material taken for the test.

APPENDIX J

[*Table* 1, *Item* (viii)]

DETERMINATION OF SULPHUR DIOXIDE

J-1. APPARATUS

J-1.1 The apparatus, assembled as shown in Fig. 3, may be used. The apparatus consists of a round-bottom resistance glass flask of 750-ml capacity fitted with a three-holed rubber stopper *D*. The rubber stopper *D* is fitted with the delivery tube *B*, the dropping funnel *E* and the sloping, water-cooled reflux condenser *F* the lower end of which is cut off at an angle. The free end of the delivery tube *B* is connected to the wash bottle *A* containing sodium carbonate solution. The upper end of the reflux condenser *F* is connected to the delivery tube *H* by the rubber stopper *G*. The free end of the delivery tube *H* nearly reaches the bottom of the 100-ml Erlenmeyer flask *J* containing 25 ml of hydrogen peroxide solution. The Erlenmeyer flask *J* is provided with a two-holed rubber stopper; through one hole passes the delivery tube *H* and, through the other, tube *K*. The free end of the tube *K* is connected to the Peligot tube *L* containing 5 ml of hydrogen peroxide solution.

J-2. REAGENTS

J-2.1 Sodium Carbonate Solution — 10 percent (w/v), aqueous.

J-2.2 Bromophenol Blue Indicator Solution — Dissolve 0·1 g of bromophenol blue in 3·0 ml of 0·05 N sodium hydroxide solution and 5 ml of ethyl alcohol (90 percent by volume) by warming gently. Make up the volume of the solution with ethyl alcohol (20 percent by volume) to 250 ml in a graduated flask.

J-2.3 Hydrogen Peroxide Solution — Dilute a 30 percent (w/v) hydrogen peroxide solution with about twice its volume of water and neutralize the free sulphuric acid that may be present in the hydrogen peroxide solution with barium hydroxide solution, using bromophenol blue indicator solution. Allow the precipitate of barium sulphate to settle, filter and determine the concentration of hydrogen peroxide in the filtrate by titrating it with standard potassium permanganate solution. Dilute the filtrate with cold water so as to obtain a 3 percent (w/v) solution of hydrogen peroxide.

J-2.4 Concentrated Hydrochloric Acid — Sp gr 1·16 (conforming to IS : 265-1950*)

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*Specification for hydrochloric acid.

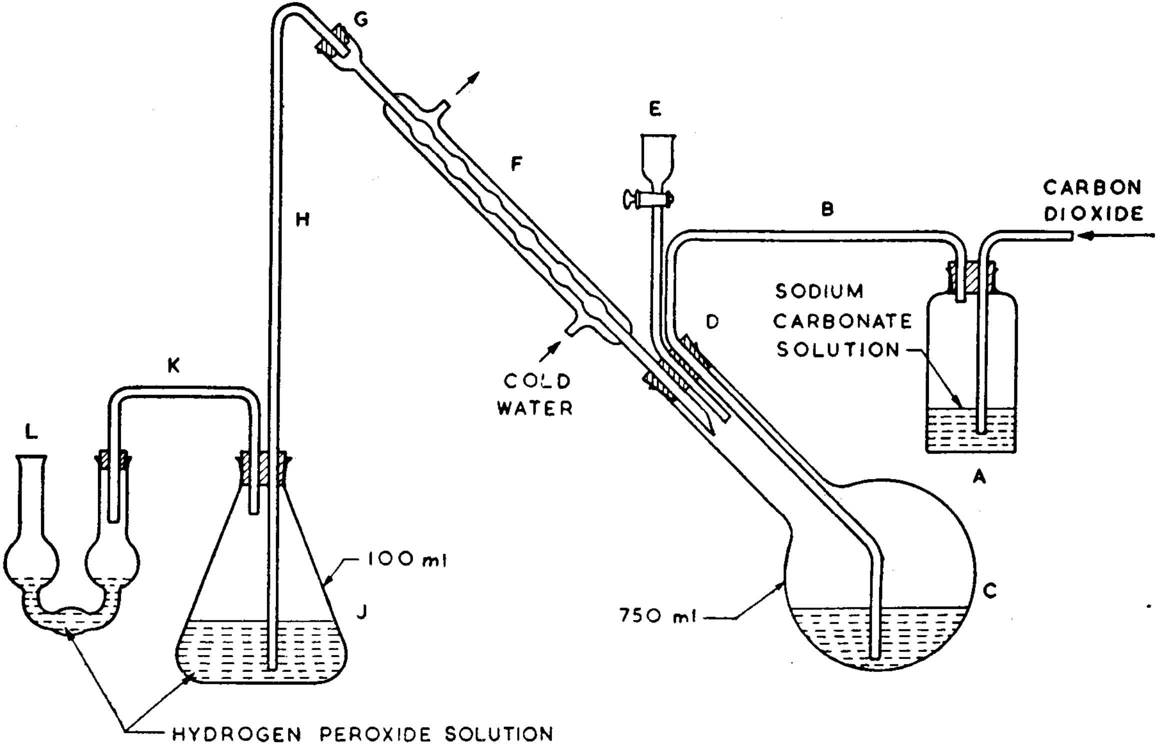

FIG. 3 ASSEMBLY OF APPARATUS FOR THE DETERMINATION OF SULPHUR DIOXIDE

J-2.5 Carbon Dioxide Gas — From a cylinder.

J-2.6 Standard Sodium Hydroxide Solution — 0·1 N standardized at the time of the experiment, using bromophenol blue indicator solution.

J-3. PROCEDURE

J-3.1 With 25 ml of hydrogen peroxide solution in the Erlenmeyer flask *J* and 5 ml in the Peligot tube *L* assemble the apparatus as shown in Fig. 3. Introduce into the flask *C*, 300 ml of water and 20 ml of concentrated hydrochloric acid through the dropping funnel *E*. Run a steady current of cold water through the condenser *F*. To expel air from the system, boil the mixture contained in the flask *C* for a short time in a current of carbon dioxide gas previously passed through the wash bottle *A*. Weigh accurately about 100 g of the material and dissolve it in the minimum quantity of water. Introduce this solution into the flask *C* through the dropping funnel *E*. Wash the dropping funnel with a small quantity of water and run the washing into the flask *C*. Distil the mixture contained in the flask *C* in a slow current of carbon dioxide gas (passed previously through the wash bottle *A*) for one hour. Just before the end of the distillation, stop the flow of water in the condenser. (This causes the condenser to become hot and drives off the residual traces of sulphur dioxide retained in the condenser.) When the delivery tube *H*, just above the Erlenmeyer flask *J*, becomes hot to touch, disconnect the stopper *G* immediately. Wash the delivery tube *H* and the contents of the peligot tube *L* with water into the Erlenmeyer flask *J*. Cool the contents of the Erlenmeyer flask to room temperature, add a few drops of bromophenol blue indicator solution and titrate with the standard sodium hydroxide solution. (Bromophenol blue is unaffected by carbon dioxide and gives a distinct colour change in cold hydrogen peroxide solution).

J-3.2 Carry out a blank determination, using 20 ml of concentrated hydrochloric acid diluted with 300 ml of water.

J-4. CALCULATION

J-4.1 Sulphur dioxide content of the material, parts per million $= \dfrac{32\,000\,(V - v)\,N}{W}$

where

V = volume in ml of the standard sodium hydroxide solution required for the test with the material,

v = volume in ml of the standard sodium hydroxide solution required for the blank determination,

N = normality of the standard sodium hydroxide solution, and

W = weight in g of the material taken for the test.

APPENDIX K

[*Table* 1, *Item* (ix)]

DETERMINATION OF PROTEOLYTIC ACTIVITY

K-1. REAGENTS

K-1.1 Cysteine Hydrochloride — 5 percent solution at *p*H 7·0.

K-1.2 Standard Sodium Hydroxide Solution — 0·1 N.

K-1.3 Casein Solution — 4 percent at *p*H 7·0.

K-2. PROCEDURE

K-2.1 Weigh accurately 0·5 g of the sample and triturate with 10 ml of cysteine hydrochloride solution and dilute to 100 ml with water. Put 30 ml of water in each of the two flasks, and then to each of them add 15 ml of casein solution and heat over a water-bath maintained at 60° C. To one flask add 5 ml of solution containing the sample and to the second flask add 5 ml of the same solution previously boiled and cooled. Maintain the flask at 60°C for 30 minutes, cool rapidly to room temperature and add to each flask 0·75 ml of phenolphthalein indicator solution and 10 ml of formaldehyde, previously neutralized to phenolphthalein. Titrate both solutions with standard sodium hydroxide solution to a definite pink colour. The proteolytic activity is expressed as number of ml of 0·1 N sodium hydroxide solution used to neutralize the solution.

SCORE SHEET FOR INDIVIDUAL JUDGE

Sample No. ....
Date of Sampling ....

DETAILS OF THE SAMPLE:

a) Product .... b) Name of Manufacturer .... c) Type ....
d) Batch No. .... e) Date of Manufacture ....

Factors	Score Points		Sample Containers										
			1	2	3	4	5	6	7	8	9	10	
Colour and Texture	Grade 1	19 — 25											
	Grade 2	16 — 18											
Taste and Flavour	Grade 1	37 — 50											
	Grade 2	33 — 37											
Absence of Defects	Grade 1	19 — 25											
	Grade 2	16 — 18											

Signature of the Judge ....
Date ....

SCORE CARD

Sample No. ....
Date of Sampling ....

DETAILS OF THE SAMPLE CONTAINER:

a) Product .... b) Name of Manufacturer .... c) Type ....
d) Batch No. .... e) Date of Manufacture ....

Factor→	Colour and Texture					Taste and Flavour					Absence of Defects					Total Scores					Average Score for				Grade of the Container
Judge→ Container Number↓	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	Colour and Texture	Taste and Flavour	Absence of Defects	Total	

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